NANOBEADS FORMATION FOR ANTICANCER DRUG RELEASE CONTROLLED BY ELECTROSPINNING

MUHAMMAD SYAFIQ BIN ABD RAHMAN

UNIVERSITI MALAYSIA PAHANG

NANOBEADS FORMATION FOR ANTICANCER DRUG RELEASE CONTROLLED BY ELECTROSPINNING

MUHAMMAD SYAFIQ BIN ABD RAHMAN

A thesis submitted in partial fulfillment of the requirement for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

FEBRUARY 2013

NANOBEADS FORMATION FOR ANTICANCER DRUG RELEASE CONTROLLED BY ELECTROSPINNING

ABSTRACT

Anticancer drug nowadays have become one of the most important industry in the world. In order to improve drug delivery, the used of nanoparticles is the main purpose for this research study. The objectives for this study are to investigate the morphology of nanoparticles produced based on different molecular weight of and concentration of Polyethylene Oxide (PEO). In this research, a very high molecular weight (MR) PEO is used as the core polymer. Two types of PEO used are with 400 000 MW and 900 000 MW. There are two types of solvents used that are distilled water and ethanol. Whey protein is used because it containing Cysteine, one of the sulphur-containing amino acid and has same properties as anticancer drug. Henceforth, whey protein is used because it is easily to purchase and cheaper. PEO acts as shell that encapsulated the drug. There are two main processes for the nanoparticles preparation that are solution preparation and electrospinning. Four types of solution are prepared; PEO with water, PEO with Ethanol, PEO with protein, and PEO with protein and ethanol. All samples are prepared till 10 mL and 20 mL and then go through the electrospinning about 5 hours. After electrospinnig the samples will be analyzed under Field Emission Scanning Electron Microscopy (FESEM) to observe the morphology of nanoparticles and Energy Dispersive X- Ray (EDX) for sulphur determination. The resulted obtained showed the lower the viscosity, the more the formation of nanobeads. Lower molecular weight also affects the nanobeads formation as lower molecular weight is more beads than higher molecular weight.

NANOBEADS FORMATION FOR ANTICANCER DRUG RELEASE CONTROLLED BY ELECTROSPINNING

ABSTRAK

Ubat antikanser pada masa kini telah menjadi salah satu industri yang paling penting di dunia. Dalam usaha untuk meningkatkan penyampaian ubat, penggunaan nanopartikel menjadi tujuan utama kajian ini dibuat. Objektif kajian ini adalah untuk menyiasat morfologi nanopartikel yang dihasilkan berdasarkan berat molekul yang berlainan dan kepekatan Oksida Polyethylene (PEO). Dalam kajian ini, berat molekul yang sangat tinggi (MR) PEO digunakan sebagai polimer teras. Dua jenis PEO digunakan dengan 400 000 MW dan 900 000 MW. Terdapat dua jenis pelarut yang digunakan iaitu air suling dan etanol. Whey protein digunakan kerana ia mengandungi Cysteine, salah satu daripada asid amino yang mengandungi sulfur dan mempunyai ciri-ciri yang sama sebagai ubat antikanser. Seterusnya, protein whey digunakan kerana ia adalah mudah dibeli dan murah. Terdapat dua proses utama untuk penyediaan nanopartikel iaitu penyediaan larutan dan electrospinning. Empat jenis larutan disediakan; PEO dengan air, PEO dengan Etanol, PEO dengan protein, dan PEO dengan protein dan etanol. Semua sampel disediakan antara 10 mL hingga 20 mL dan kemudian akan melalui electrospinning kira-kira 5 jam. Selepas electrospinnig, sampel akan dianalisis di bawah Pelepasan Lapang Mengimbas Mikroskopi Elektron (FESEM) untuk melihat morfologi nanopartikel dan Sebaran Tenaga X-Ray (EDX) bagi menentukan sulfur. Keputusan yang diperolehi menunjukkan lebih rendah kelikatan, lebih banyak pembentukan nanopartikel. Berat molekul yang lebih rendah juga memberi kesan kepada pembentukan nanopartikel iaitu apabila berat molekul yang rendah adalah menghasilkan lebih banyak manik daripada berat molekul yang lebih tinggi.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

C	Carbon
DC	Direct Current
DDS	Drug Delivery System
EDX	Energy Dispersion X-Ray
Etc	Et cetera
Etoh	Ethanol
FE-SEM	Field Emission Scanning Electron Microscope
K	Potassium
MW	Molecular Weight
Na	Sodium
NPs	Nanoparticles
PEO	Polyethylene
S	Sulphur
Si	Silicone

LIST OF SYMBOLS

%	Percentage
M	Micro
G	Gauge
h	hour
k	kilo
m	Meter
ml	mililiter
mm	millimeter
MPa	Mega Pascal
Nm	Nanometer
RPM	Revolution Per Minutes
s	second
S	Siemens (unit of conductivity)
V	Voltage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cancer is a disease in which the control of growth is lost in one or more cells, leading either to a solid mass of cells known as a tumour or to a liquid cancer .It is one of the leading causes of death throughout the world, in which the main treatments involve surgery, chemotherapy, and radiotherapy (Nussbaumer *et al*.,2011). Nanotechnology, which deals with features as small as a 1 billionth of a meter, began to enter into mainstream physical sciences and engineering some 20 years ago. Lamprecht (2009) describes the use of nanoparticulate drug delivery systems as one important aspect in the newly developing field of nanomedicine to allow innovative therapeutic approaches.

Nanotechnology as a delivery device has a very promising application in drug delivery as it has advantages to enhance drug transport across biological barriers and deliver the drug at selective or targeted tissue or organ. As the principle of drug targeted is to reduce the total amount of drug administration, ongoing efforts have

been made over the past decade to develop systems or drug carriers that are capable of delivering the active molecules specifically to the target organs to increase the therapeutic efficacy. The site-specific delivery systems will allow an effective drug concentration to be maintained for a longer time interval and decrease the side effects. Recent applications of nanoscience include the use of nanoscale materials in electronics, catalysis, and biomedical research. Among these applications, strong interest has been shown to biological processes such as blood coagulation control and multimodal bioimaging, which has brought about a new and exciting research field called nanobiotechnology (Suh *et al*., 2009).

Nanoparticulate delivery systems are extensively investigated as a drug delivery strategy in the pharmaceutical research. In general, nanocarriers may protect a drug from degradation, enhance drug absorption by facilitating diffusion through epithelium, modify pharmacokinetic and drug tissue distribution profile and improve intracellular penetration and distribution (Elzoghby *et al*., 2012). Nanoparticles provide a new mode of cancer drug delivery functioning as a carrier for entry through fenestrations in tumour vasculature allowing direct cell access. Different types of nano-sized carriers, such as polymeric nanoparticles, solid lipid nanoparticles, ceramic nanoparticles, magnetic nanoparticles, polymericmicelles, polymer-drug conjugates, nanotubes, nanowires, nanocages and dendrimers, are being developed for various drug-delivery applications

Polymeric nanoparticles can be fabricated from polysaccharides proteins and synthetic polymers (Elzoghby *et al*., 2012). Among the available potential colloidal drug carrier systems, protein-based nanoparticles are particularly interesting as they hold certain advantages such as greater stability during storage and in vivo, being non-toxic and non-antigenic and their ease to scale up during manufacture over other

drug delivery systems (Elzoghby *et al*., 2012). The efficiency of drug delivery to various parts of the body is directly affected by particle size. Nanostructure mediated drug delivery, a key technology for the realization of nanomedicine, has the potential to enhance drug bioavailability, improve the timed release of drug molecules, and enable precision drug targeting (Hughes, 2005).

Electrospinning is a technique that can easily fabricate nanofiber, nanobeads and microfiber meshes from different types of polymer. Due to their unique features such as high surface-to-volume ratio, morphological design flexibility and extracellular matrices structure-like, nanoparticles are used as scaffolds for drug delivery and tissue engineering. Low molecular weight drugs and biomolecules such as proteins and nucleic acids can be encapsulated into the electrospun fibers (Xu *et al*., 2008).

1.2 Problem Statement

Most of the drugs used nowadays give side effect to patient due to toxicity and lack of therapeutic ethic. Moreover, the efficiency toward cancer cell also is still in lower form. Henceforth, in order to solve this problem, the used of nanotechnology have become more important for cancer treatment. According to Kim and Pack (2006), a wide variety of new, more potent and specific therapeutics are being created in advances in biotechnology. A drug delivery system is designed to provide a therapeutic agent in the needed amount, at the right time and to the proper location in the body in a manner that optimizes the efficacy, increases compliance and minimizes side effects. Due to common problems in drug delivery

such as low solubility, high potency and poor stability, it can impact the efficiency and potential of the drug itself. Thus, there is a corresponding need for safer and more effective methods and devices for drug delivery.

Biodegradable nanoparticles are frequently used to improve the therapeutic value, improving bioavailability, solubility, and retention time. It also helps to reduces expenses for the treatment and risk of toxicity.

1.3 Objectives

1.3.1 To study the effect of PEO of different molecular weight toward the formation of nanobeads.

1.3.2 To study the effect of solution viscosity on the nanobeads formation.

1.4 Scope of Study

1.4.1 To study the effect of different molecular weight of Polyethylene Oxide (PEO) on nanobeads formation.

1.4.2 To study the effect on nanobeads formation by using different concentration of PEO that will affect the viscosity

1.5 Rationale of Significant Study

The significance of this research is to identify the formation of nanoparticles using electrospinning of different concentration and viscosity of solution. By using nanobaeads membrane encapsulated with drug as device, the drug can be released at controlled rates for a long period of time. The advantages of employing such systems are the drug release rates can be designed to the needs of a specific application. Apart from that, controlled drug delivery systems may provide drug protections especially proteins that are easily destroyed by the body. Controlled drug delivery systems can also increase patient comfort and compliance by substituting frequent doses (daily injectibles) with infrequent injection (once a month injection or less).

CHAPTER 2

LITERATURE REVIEW

2.1 Electrospinning

2.1.1 History of Electrospinning

Elcetrospinning is a technique that used very high voltage power supply to produce nanofibers. It also used to produce nanobeads using certain variation in parameter. According to Liang *et al*., (2007), the word electrospinning is derived from electrostatic spinning. This technique has been used recently since 1994. However, Liang *et al*., (2007) stated that electrospinning was first observed in 1897 by Rayleigh.

The popularity of this technique can be approved by the statistic and fact that over 200 universities and research institutes from around the world are studying electrospinning as claimed by Zussman *et al*., (2003). There are various aspects of the electrospinning process and the fiber it produces and also the number of patents for applications based on electrospinning has grown in recent years. Some companies

such as eSpin Technologies, NanoTechnics, and KATO Tech are actively engaged in reaping the benefits of the unique advantages offered by electrospinning. Meanwhile, companies such as Donaldson Company and Freudenberg have been using this process for the last two decades in their air filtration products (Ramakrishna *et al*., 2006).

This electrospinning is broadly used technology for electrostatic fiber formation which uses electrical forces to produce electropun fibers with diameters ranging from 2nm to several micrometers. It uses polymer solutions of both natural and synthetic polymers. This technique seems to be increasingly applied in research and had take the commercial attention over the past decade according to Ahn *et al*., (2006).As claimed by Zussman *et al*., (2003), this process offers unique capabilities and advantages for producing novel natural nanofibers and fabrics with controllable pore structure.

According to Reneker *et al*., (2000) this process of electrospinning has gained a lot of attention over the world. This phenomenon in the last decade not only due to its versatility in spinning a wide variety of polymeric fibers, but also its capability in producing consistent fibers and nanobeads in the submicron range consistently.This consistency is otherwise difficult to achieve by using standard mechanical fiber-spinning technologies techniques (Reneker *et al*.,2000). According to Luu *et al*., (2003), electrospun fibers have been successfully applied in various fields, such as, nanocatalysis, tissue engineering scaffolds, protective clothing, filtration, biomedical, pharmaceutical, optical electronics, healthcare, biotechnology, defense and security, and environmental engineering because of the smaller pores and higher surface area than regular beads.

Overally, this electrospinning is relatively simple and effective to produce nanobeads from a variety of polymers. Although it is more suitable to produce nanofiber, but nanobeads formation are also possible using electrospinning as claimed bt Liang *et al*., (2007). Spun nanobeads also offer some advantages such as high surface-to-volume ratio, tunable porosity, malleability to conform to a wide variety of sizes and shapes (Liang *et al*., 2007). Hence forth, Liang *et al*., (2007) also claimed that because of these advantages, electrospun nanofibers have been widely investigated in the past several years. Those advantages are probably had been applied to several process such as filtration, optical and chemical sensors, electrode materials and biological scaffolds (Liang *et al*., 2007).

2.1.2 Electrospinning Process

Electrospinning , a spinning technique that used unique approach that is by using electrostatic forces to produce fine electrospun from polymer solutions. The electrospun produced have a thinner diameter (from nanometer to micrometer) as stated by Kidoaki *et al*., (2005). Moreover, a very high voltage supply a range of several tens of kVs is necessary for the process. According to Chew *et al*., (2006a), various techniques such as electrostatic precipitators and pesticide sprayers work similarly to the electrospinning process and this process, mainly based on the principle that strong mutual electrical repulsive forces overcome weaker forces of surface tension in the charged polymer liquid. Based on the Kidoaki *et al*., (2005), there are two types of electrospinning that had been used nowadays. The two types of electrospinning are vertical and horizontal. With the expansion of this technology,

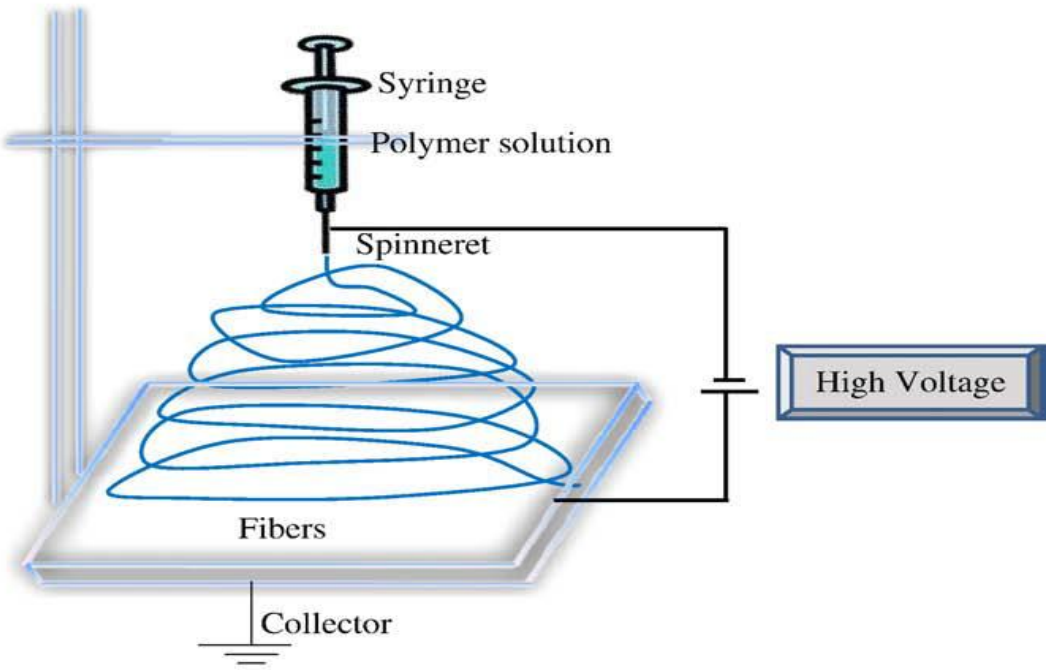
several research groups have developed more sophisticated systems that can fabricate more complex nanofibrous structures in a more controlled and efficient manner.

This process of electrospinning should be running at room temperature with standard atmosphere conditions. The standard set up of electrospinning apparatus is shown in Figure 2.1 (a and b). The standard electrospinning process consists of three major components that are a high voltage power supply, a spinneret and a grounded collecting plate and utilizes a high voltage source to inject charge of a certain polarity into a polymer solution or melt, which is then accelerated towards a collector of opposite polarity (Liang *et al*., 2007).

According to the article from Huang *et al*., (2003), they claimed that most of the polymers are dissolved in some solvents before electrospinning, and when it completely dissolves forms polymer solution. The polymer fluid is then introduced into the capillary tube for electrospinning. However, Huang *et al*., (2003) also claimed that some polymers may emit unpleasant or even harmful smells, so the processes should be conducted within chambers having a ventilation system. In the electrospinning process, a polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field and an electric charge is induced on the liquid surface due to this electric field. When the electric field applied reaches a critical value, the repulsive electrical forces overcome the surface tension forces.

Eventually, a charged jet of the solution is ejected from the tip of the Taylor cone and an unstable and a rapid whipping of the jet occurs in the space between the capillary tip and collector which leads to evaporation of the solvent, leaving a polymer behind (Milasius, 2007). The jet is only stable at the tip of the spinneret and after that instability starts. Thus, the electrospinning process offers a simplified technique for fiber formation.

a)



b)

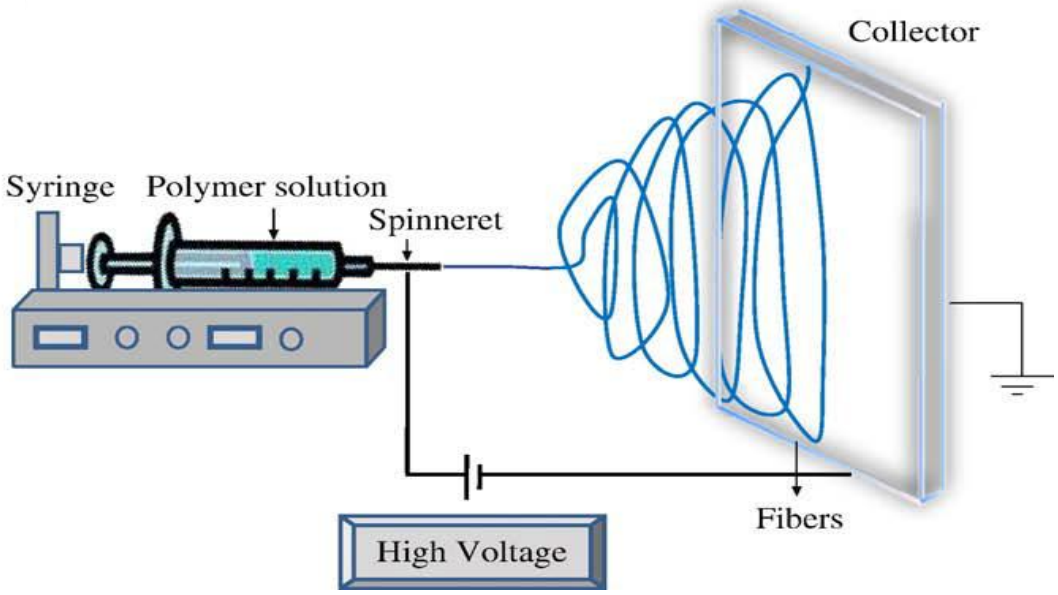


Figure 2.1 Schematic diagram of set up of electrospinning apparatus (a) typical vertical set up and (b) horizontal set up of electrospinning apparatus. (Source: Parven *et al*, 2012)

2.2 Drug Delivery Technology

Researches on drug delivery system (DDS) have many benefits on pharmaceutical industry nowadays. According to Swatantra *et al*., (2012), some of the benefits are include improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved targeting for a specific site to reduce unwanted side effects. Those researches also state about the challenge for both drug and drug delivery companies is to deliver both existing and emerging drug technologies in a manner that improves the benefits to the patients.

Over the past several years, great advances have been made on development of novel drug delivery systems of anticancer drug. According to Ajazuddin *et al*., (2010), in phyto-formulation research, developing nanodosage have a number of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation. Some of the examples for the nanodosage polymeric are nanoparticles and nanocapsules, liposomes, solid lipid nanoparticles, phytosomes and nanoemulsion (Ajazuddin *et al*., 2010).

Injectable polymers that have biocompatibility and biodegradability are important biomaterials for drug delivery system (DDS) and tissue engineering (Swatantra *et al*., 2012). Accoring to Medina *et al*., (2004), multiple synthetic and natural biodegradable polymers have been investigated for these purposes, including polyesters, polyethers, poly amino-acids, polysaccharides, and proteins. These polymers are employed as injectable drug delivery system, and especially as

injectable drug delivery system for cancer chemotherapy, and have been investigated actively so as to minimize the toxic side effects and increase the carcinostatic pharmaceutical effects (Heller *et al*., 2004). Methods of local administration of drug delivery system, nanoparticles, microspheres, polymeric micelles, liposomes, and hydrogel systems, for targeting and controlled release have been investigated with nonand biodegradable polymers. However, the targeting drug delivery system has not been satisfactorily achieved.

2.3 Nanoparticles in Drug Delivery

Drug delivery is an interdisciplinary and independent field of research and is gaining the attention of pharmaceutical researchers, medical doctors and industry as stated by Singh et al., (2009). A safe and targeted drug delivery could improve the performance of some classic medicines already on the market, and moreover, will have implications for the development and success of new therapeutic strategies such as anticancer drug delivery, peptide and protein delivery and gene therapy. According to Parveen et al., (2012) several drug-delivery technologies have emerged and a fascinating part of this field is the development of nanoscale drug delivery devices in the last decade.

Nanoparticles (NPs) have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and more recently, nucleotides. NPs and other colloidal drug-delivery systems modify the kinetics, body distribution and drug release of an associated drug. This review article focuses on the potential of nanotechnology in medicine and discusses different nanoparticulate drug-delivery

systems including polymeric NPs, ceramic NPs, magnetic NPs, polymeric micelles and dendrimers as well as their applications in therapeutics, diagnostics and imaging (Parveen *et al*., 2012). The applications of nanotechnology in various disciplines and specifically in healthcare are becoming increasingly common and the process of replacing traditional medicines has already begun. Thus, although efficient drug delivery is one of the most prominent problems faced by the biotechnological and pharmaceutical industries, nanotechnology can promote the innovative utilization of the myriad existing drugs produced by these industries.

Nanotechnology focuses on formulating therapeutic agents in biocompatible nanocarriers, such as NPs, nanocapsules, micellar systems and dendrimers (Figure 2.2). Moreover, one of the major advantages that nanotechnology offers is targeted drug delivery to the site of disease. This can be achieved either through passive targeting of drugs to the site of action or by active targeting of the drug (Parveen *et al*., 2012)